

WHAT IS CLAIMED IS:

1 ~~X~~. A protein released by a mammalian fetal trophoblast cell or a
2 chorionic villus wherein the level of release is substantially changed when the
3 cell or villus is grown under hypoxic conditions characterized by a partial
4 pressure of oxygen (pO_2) of 14 mm of mercury (mm Hg), wherein said protein
5 is selected from the group of proteins consisting of:

6 (a) Protein A having a molecular weight of about 21 kDa and a
7 pI of 6.0 wherein the release of said protein, under hypoxic conditions is
8 increased;

9 (b) Protein B having a molecular weight of about 22 kDa and a pI
10 of 7.0 wherein the release of said protein, under hypoxic conditions is
11 increased;

12 (c) Protein C having a molecular weight of about 23 kDa and a pI
13 of 7.5 wherein the release of said protein, under hypoxic conditions, is
14 increased;

15 (d) Protein D having a molecular weight of about 55 kDa and a
16 pI of 8.5 wherein the release of said protein, under hypoxic conditions, is
17 increased;

18 (e) Protein E having a molecular weight of about 62 kDa and a pI
19 of 5.5 wherein the release of said protein, under hypoxic conditions, is
20 increased;

21 (f) Protein F having a molecular weight of about 40 kDa and a pI
22 of 4.5 wherein the release of said protein, under hypoxic conditions, is
23 decreased;

24 (g) Protein G having a molecular weight of about 67 kDa and a
25 pI of 6.5 wherein the release of said protein, under hypoxic conditions, is
26 decreased;

27 (h) Protein H having a molecular weight of about 75 kDa and a
28 pI of 9.0 wherein the release of said protein, under hypoxic conditions, is
29 decreased;

30 (i) A protein of spot number 2 comprising an amino acid
31 sequence selected from the group consisting of sequence 1, and sequence 2 as
32 shown in Table 2;

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4/18/95 (priority doc.)

(j) A protein of spot number 3 comprising an amino acid sequence selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence sequence 12, and sequence 13 as shown in Table 2;

(n) A protein of spot number 11 comprising an amino acid sequence selected from the group consisting of sequence 14, sequence 15, sequence 16, sequence 17, and sequence 18 as shown in Table 2; and

(o) A protein of spot number 20 comprising an amino acid sequence selected from the group consisting of sequence 21, and sequence 22 as shown in Table 2; and

(p) A human apolipoprotein A-1.

2. A protein of claim 1, wherein the protein is selected from the group consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions, is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions, is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased; and,

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(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased.

3. A protein of claim 1, wherein the protein is selected from the group consisting of:

(f) Protein F having a molecular weight of about 40 kDa and a pI of 4.5 wherein the release of said protein, under hypoxic conditions, is decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased; and

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased.

4. A protein of claim 1, wherein the protein is selected from the group consisting of:

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(j) A protein of spot number 3 comprising an amino acid sequences selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

17 (m) A protein of spot number 10 comprising an amino acid
18 sequence selected from the group consisting of sequence 12, and sequence 13
19 as shown in Table 2 and wherein the release of said protein, under hypoxic
20 conditions, is increased;

21 (n) A protein of spot number 11 comprising an amino acid
22 sequence selected from the group consisting of sequence 14, sequence 15,
23 sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein
24 the release of said protein, under hypoxic conditions, is decreased; and

25 (o) A protein of spot number 20 comprising an amino acid
26 sequence selected from the group consisting of sequence 21, and sequence 22 as
27 shown in Table 2 and wherein the release of said protein, under hypoxic
28 conditions, is increased; and

29 (p) A human apolipoprotein A-1 wherein the release of said
30 protein, under hypoxic conditions, is increased.

1 ~~5. A method of culturing human fetal trophoblast cells or~~
2 ~~chorionic villi under hypoxic conditions, said method comprising the step of~~
3 ~~culturing the trophoblast cells or chorionic villi under an atmosphere comprising~~
4 ~~less than about 20% oxygen.~~

5 6. A method of claim 5, wherein the method further comprises
6 measuring the release of a protein selected from the group consisting of:

7 (a) Protein A having a molecular weight of about 21 kDa and a
8 pI of 6.0 wherein the release of said protein, under hypoxic conditions, is
9 increased;

10 (b) Protein B having a molecular weight of about 22 kDa and a pI
11 of 7.0 wherein the release of said protein, under hypoxic conditions, is
increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI
of 7.5 wherein the release of said protein, under hypoxic conditions, is
increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased;

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased;

(f) Protein F having a molecular weight of about 40 kDa and a pI of 4.5 wherein the release said protein, under hypoxic conditions, is decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased; and

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased;

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(j) A protein of spot number 3 comprising an amino acid sequences selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence 12, and sequence 13 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

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44 (n) A protein of spot number 11 comprising an amino acid
45 sequence selected from the group consisting of sequence 14, sequence 15,
46 sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein
47 the release of said protein, under hypoxic conditions, is decreased; and

48 (o) A protein of spot number 20 comprising an amino acid
49 sequence selected from the group consisting of sequence 21, and sequence 22 as
50 shown in Table 2 and wherein the release of said protein, under hypoxic
51 conditions, is increased; and

52 (p) A human apolipoprotein A-1 wherein the release of said
53 protein, under hypoxic conditions, is increased.

1 7. A method of detecting hypoxic cytotrophoblast cells or
2 hypoxic chorionic villi, said method comprising measuring the release of a
3 protein selected from the group consisting of:

4 (a) Protein A having a molecular weight of about 21 kDa and a
5 pI of 6.0 wherein the release of said protein, under hypoxic conditions, is
6 increased;

7 (b) Protein B having a molecular weight of about 22 kDa and a pI
8 of 7.0 wherein the release of said protein, under hypoxic conditions, is
9 increased;

10 (c) Protein C having a molecular weight of about 23 kDa and a pI
11 of 7.5 wherein the release of said protein, under hypoxic conditions, is
12 increased;

13 (d) Protein D having a molecular weight of about 55 kDa and a
14 pI of 8.5 wherein the release of said protein, under hypoxic conditions, is
15 increased;

16 (e) Protein E having a molecular weight of about 62 kDa and a pI
17 of 5.5 wherein the release of said protein, under hypoxic conditions, is
18 increased;

19 (f) Protein F having a molecular weight of about 40 kDa and a pI
20 of 4.5 wherein the release of said protein, under hypoxic conditions, is
21 decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased; and

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased;

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(j) A protein of spot number 3 comprising an amino acid sequences selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence 12, and sequence 13 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(n) A protein of spot number 11 comprising an amino acid sequence selected from the group consisting of sequence 14, sequence 15, sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased; and

(o) A protein of spot number 20 comprising an amino acid sequence selected from the group consisting of sequence 21, and sequence 22 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased; and

54 (p) A human apolipoprotein A-1 wherein the release of said
55 protein, under hypoxic conditions, is increased;
56 wherein the release of the protein is increased or decreased
57 relative to identical cells grown under identical culture conditions but under
58 normal oxygen conditions.

1 8. A method of claim 7, wherein the measurement is by direct
2 determination of the protein.

3 9. A method of claim 7, wherein the determination comprises
4 the step of binding an antibody to the protein and determining the quantity of
5 bound antibody present in a sample relative to the quantity of antibody bound to
6 protein obtained from normoxic trophoblasts or normoxic chorionic villi.

7 10. A method of claim 7, wherein the determination comprises
8 detecting mRNA encoding any of the proteins and determining if the level of
9 mRNA has changed relative to similarly treated normoxic cells.

10 11. A method for detecting an abnormal placental function by
11 analysing a biological sample from a pregnant mammal for abnormal release of
12 a protein selected from the group consisting of:

13 (a) Protein A having a molecular weight of about 21 kDa and a
14 pI of 6.0 wherein the release of said protein, under hypoxic conditions, is
15 increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI
of 7.0 wherein the release of said protein, under hypoxic conditions, is
increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI
of 7.5 wherein the release of said protein, under hypoxic conditions, is
increased;

(d) Protein D having a molecular weight of about 55 kDa and a
pI of 8.5 wherein the release of said protein, under hypoxic conditions, is
increased;

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1 (e) Protein E having a molecular weight of about 62 kDa and a pI
2 of 5.5 wherein the release of said protein, under hypoxic conditions, is
3 increased;

4 (f) Protein F having a molecular weight of about 40 kDa and a pI
5 of 4.5 wherein the release of said protein, under hypoxic conditions, is
6 decreased;

7 (g) Protein G having a molecular weight of about 67 kDa and a
8 pI of 6.5 wherein the release of said protein, under hypoxic conditions, is
9 decreased; and,

10 (h) Protein H having a molecular weight of about 75 kDa and a
11 pI of 9.0 wherein the release of said protein, under hypoxic conditions, is
12 decreased;

13 (i) A protein of spot number 2 comprising an amino acid
14 sequence selected from the group consisting of sequence 1, and sequence 2 as
15 shown in Table 2 and wherein the release of said protein, under hypoxic
16 conditions, is decreased;

17 (j) A protein of spot number 3 comprising an amino acid
18 sequences selected from the group consisting of sequence 3, sequence 4,
19 sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said
20 protein, under hypoxic conditions, is decreased;

21 (k) A protein of spot number 5 comprising amino acid sequence
22 number 7 as shown in Table 2 and wherein the release of said protein, under
23 hypoxic conditions, is increased;

24 (l) A protein of spot number 7 comprising amino acid sequence
25 number 8 as shown in Table 2 and wherein the release of said protein, under
26 hypoxic conditions, is increased;

27 (m) A protein of spot number 10 comprising an amino acid
28 sequence selected from the group consisting of sequence 12, and sequence 13
29 as shown in Table 2 and wherein the release of said protein, under hypoxic
30 conditions, is increased;

31 (n) A protein of spot number 11 comprising an amino acid
32 sequence selected from the group consisting of sequence 14, sequence 15,

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1 sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein
2 the release of said protein, under hypoxic conditions, is decreased; and

3 (o) A protein of spot number 20 comprising an amino acid
4 sequence selected from the group consisting of sequence 21, and sequence 22 as
5 shown in Table 2 and wherein the release of said protein, under hypoxic
6 conditions, is increased; and

7 (p) A human apolipoprotein A-1 wherein the release of said
8 protein, under hypoxic conditions, is increased..

1 12. A method of claim 11, wherein said abnormal placental
2 function is a symptom of a disease of pregnancy selected from the group
3 consisting of threatened abortion, intrauterine growth retardation, gestational
4 trophoblast diseases including molar pregnancy, choriocarcinoma, placental site
5 tumors, ectopic pregnancy, proteinuria, pregnancy induced hypertension and
6 preeclampsia.

1 13. A method of claim 12, wherein said disease of pregnancy is
2 preeclampsia.

1 14. A method of screening for agents that mitigate the effects of
2 an abnormal maternal-placental interface, said method comprising:

3 (i) culturing cytotrophoblasts under hypoxic conditions in the
4 presence of said agent; and

5 (ii) assaying for changes in the phenotype of said hypoxic
6 trophoblasts relative to hypoxic trophoblasts cultures without the presence of
7 said agent.

1 15. The method of claim 14, wherein said assaying comprises
2 measuring the invasiveness of said trophoblasts.

1 16. The method of claim 14, wherein said assaying comprises
2 measuring the changes in the levels of release of proteins expressed by said
3 trophoblasts.

1 17. The method of modeling, *in vitro*, an abnormal maternal-
2 placental interface, said method comprising culturing trophoblast cells or
3 chorionic villi in a hypoxic environment.

1 18. The method of claim 17, wherein said hypoxic environment
2 comprises an atmosphere comprising less than about 20% oxygen.

1 19. A method for identifying proteins that are indicative of
2 metastasis said method comprising:
3 (i) raising cytotrophoblasts under hypoxic conditions; and
4 (ii) detecting proteins that demonstrate an altered release level as
5 a result of said hypoxic conditions; and,
6 (iii) determining if said proteins are present in metastatic cells.

1 20. A method of claim 19, wherein the determining is done by
2 immunoassay using antibodies specific for at least one of the proteins of step ii.

1 21. A method for identifying proteins that are indicative of an
2 abnormal maternal placental interface said method comprising:
3 (i) culturing cytotrophoblasts under hypoxic conditions; and
4 (ii) detecting proteins that demonstrate an altered release level as
5 a result of the hypoxic conditions.

1 22. A method of claim 21, wherein said abnormal maternal
2 placental interface is indicative of preeclampsia.